



Impact of A1/A2 forms of Cow's Milk on Human Health-A review

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ABSTRACT

Milk derived peptides may adversely affect the biological health of humans by increasing the risk of dependency on insulin to overcome the occurrence of diabetes. The higher occurrence of the diabetes relies on the relationship of type 1 diabetes (T1D) and the consumption of variants A1 and B beta –casein from cow's milk. The production of BCM-7 is more in A1 milk than A2 milk and the difference is basically due to position 67 of the beta casein chain. However, proline in A2 is substituted by histidine in A1 milk casein chain. Deleterious effects of A1 milk in the process of digestion in human stomach and intestines have not been reviewed earlier. It is an effort to review the aspect thoroughly and bring minor details into focus to have better understanding of the milk particularly in perspective of human health.

Keywords: A1milk, A2 milk, exotic cows, indigenous cows, proline amino acid, histidine amino acid

Bovine milk is a resource of lipids, proteins, amino acids, vitamins and minerals and it also contains several nutrients needed for growth and development not only for calf but also for humans. It contains immunoglobulins, hormones, growth factors, cytokines, nucleotides, peptides, polyamines, enzymes and several others bioactive peptides. The lipids in milk are emulsified in globules coated with membranes and proteins are in colloidal dispersions as micelles. The casein micelles are seen and observed as colloidal complexes of protein and salts, especially calcium (Keenan and Patton, 1995) where as lactose and most other minerals are in solution form. Milk composition varies with stage of lactation, age, breed, nutrition, and energy balance and health status of the udder. Colostrums also differ considerably from milk to milk and the proteins are higher in colostrums than in the later lactational milk (Ontosouka *et al.*, 2003). The change in milk composition changes with the change in the nutrients given to the growing infants during the entire lactation period. Proteins from certain specific milk are involved in the early development of immune

response and other milk proteins gets involved in the non-immunological defence (e.g. lactoferrin). Different fatty acids are present in the milk (Jensen *et al.*, 1995) and all these fatty acids constitute nutrient rich milk food.

PROTEIN

The bovine milk contains huge array of proteins from anti-microbials to hormones and enzymes to antibodies (Clare *et al.*, 2000) and contains about 32 g protein/l (USDA national nutrient database for standard, 2007) (Table 1). Milk nitrogen is profusely present in milk caesins, whey proteins and non-protein nitrogen and milk casein is around 80%. Biological function of caseins is to carry calcium and phosphate and to form a clot in the stomach for efficient digestion. The whey proteins in milk are globular proteins are water soluble and its principal fractions are beta-lactoglobulin, alpha-lactalbumin, bovine serum albumin and immunoglobulins. Whey is also been used to curdled to cheese for human consumption as ricotta and brown cheese. Whey proteins are also being used as an additive



to in bread, crackers, pastry and animal feed. In animals the consumed products after digestion are released slowly however, whey proteins get rapidly digested and appear faster in the blood post prandial plasma (Nilsson *et al.*, 2007). Milk proteins (e.g. secretory immunoglobulin A, lactoferrin, 1-antitrypsin, α -casein and lactalbumin) in human digestive tract appears as peptides or whole proteins and their functions depending upon their relative resistance towards digestive enzymes (Lonnerdal, 2007).

As several bioactive proteins and peptides derived from milk proteins are potential modulators of various regulatory processes in the body. These bioactive proteins and peptides are therefore considered as functional foods and therefore all of these needed to be produced on larger scale to meet out the human consumption demand.

Some of the mineral binding and cytomodulatory peptides from bovine milk has been regarded as safe over disease risk as they have been claimed to be as health enhancer (Meisel, 2003). Lately, it was suggested that the milk protein composition may differ among breeds (Swaigood, 1995). Few reports have claimed that the concentration of beta-casein A1 is low in milk from cows in Iceland and New Zealand and this later became the evidence of such suggestions. It was further observed by few researchers that such proteins may develop and assist in the development of diabetes and cardiac diseases (Birgisdottir, 2002). However, the same thought and claim did not sustain longer in the want of many such investigations and claims which could justify the fact related to A1 beta-casein of cow milk having such adverse effect in humans (Truswell, 2005).

MILK PEPTIDES

Milk is rich in essential amino acids and has branched chain amino acids. And all of these amino acids are having different role in human metabolism. These amino acids in addition to its role in human metabolism also serve as bait for gluconeogenesis and also help in protein synthesis (Etzel, 2004). Leucine is a branched chain amino acids have been found in milk claimed to aid in muscle protein synthesis through insulin signalling pathway (Wolfe, 2002). It has also been revealed that combination of milk with a meal with high sugar might stimulate insulin release and helps to reduce the blood glucose concentration after

meals (Frid *et al.*, 2005). It was later felt that a reduction in postprandial blood glucose is favourable and the consumption of milk would lower the risk of diseases related to insulin resistance syndrome (Pereira *et al.*, 2002). Lately, few researchers have demonstrated the association between milk consumption and individual's hypertension (Jauhiainen and Korpela, 2007). However, the reason for the same was pointed out because others claim that few milk proteins have been identified to reverse the hypertensive effects by inhibiting angiotensin-converting enzymes and also reduces thrombosis with in the blood (Jauhiainen and Korpela, 2007).

MASS SPECTROMETRIC MILK PROTEINS

Milk usually has two different types of proteins i.e. caseins and whey proteins. Caseins is about 80% (Niki *et al.*, 1994) but whey proteins constitute about 14% (McLachlan, 2001) in bovine milk. Milk proteins have been put into three categories so far according to their solubility potential 1. Caesin; 2. whey proteins; milk fat globule membrane (MFGM) proteins (Fig. 1). Casein protein is group of proteins coded by four tightly-linked autosomal genes (CSN1S1, CSN1S2, CSN2 and CSN3). They are sub-divided into four families: s1-, s2-, α - and κ -CN.6 Milk caseins are assembled into proteins and minerals macromolecules and thereby form casein micelles. The structure of the micelles shows κ -CN primarily on to the surface and protects the micelle structure from destabilization. There are variations in the quantity of CNs depending upon the species milk is being categorized. Bovine milk shows 80% (w/w) milk proteins and in women's it is 35. The alignment of the amino acid sequences differs normally but in ruminants greatest level of amino acid identities have been found (Fig. 4). But today it is all known that alignment of the amino acid sequences have considerable difference across species. Though the highest level of identities has been found in ruminants but the genetic distance between these and other species have been observed to decline (Fig. 1).

Caesin molecular mass is about 18–25 kDa and are quite heterogenous in nature as it develops through post-translational modifications and alternative splicing of the gene product and genetic polymorphisms (Caroli *et al.*, 2009; Martin *et al.*, 2002). Caesin show phosphorylation as they are basically phosphoproteins. Constrastingly,

k-CN contains carbohydrate moieties which are attached to the k-CN via O-glycosidic bonds to serine and threonine residues in the C-terminal region of the protein.

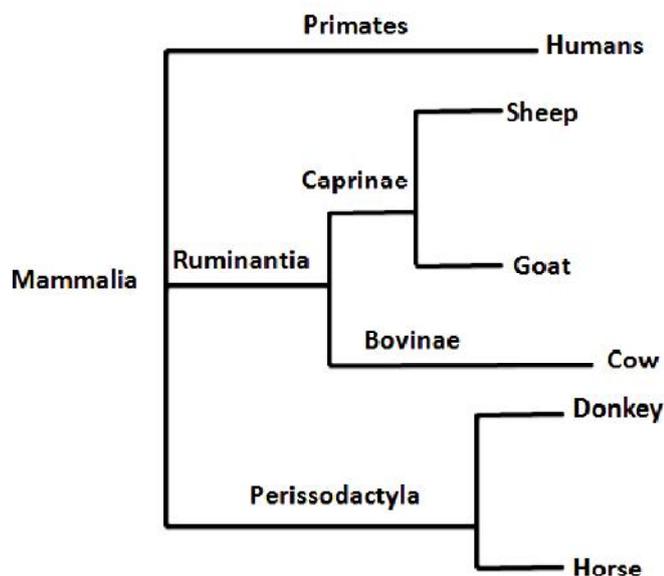


Fig. 1: Phylogenetic branching of cow compared with other mammalian species

The amino acid sequences of α s₂- and k-CN also contains cysteine residues (Holland *et al.*, 2008). Caseins doesnot have stable secondary and tertiary structures and due to which they become susceptible to proteolysis. -CN has been shown to be the most susceptible in the presence endogenous milk protease plasmin (Bastian and Brown, 1996). Plasmin cleaves at specific sites of -CNs and produces similar C-terminal and N-terminal polypeptide fragments as g-caseins and proteose peptone (PP) components, respectively (Nielsen, 2002). The components so produced found to be soluble in acidic condition of pH ~4.6. These products have been termed as whey proteins and their other components are -lactoglobulin (-LG), α -lactalbumin (-LA), serum albumin (SA), lactoferrin (LF) and immunoglobulins (Igs), and numerous minor proteins (i.e. low-abundance proteins), including enzymes, enzyme inhibitors, metal-binding proteins etc. Finally, MFGM proteins though have lower nutrient values but have been designated as milk proteins which have been found to have important effects in different cell processes and defence mechanism of the newly born. MFGM proteins has 1–4% of total protein content in mature milk.

Major MFGM proteins include mucin 1, xanthine oxidase, butyrophilin, adipophilin, PAS6/7 (lactadherin) and fatty acid binding protein (Cavaletto *et al.*, 2008).

Taurine

Taurine is around 18 mg/l and 1mg/l in human breast milk and cow's milk respectively. Goat milk is nearest to the human breast milk in composition the concentration of taurine is around 46–91 mg/l (Cataldi *et al.*, 2004). Taurine is an essential amino acid in preterm neonates and in deficient patients, diabetes patients and patients having chronic illness due to hepatic, heart and renal failure (Lourenco and Camilo, 2002; Li *et al.*, 2004). Supplementation of taurine @50mg/kg body weight routine have been documented to have benefit in humans as well as in animals (Burger *et al.*, 1992).

Taurine amino acid has been abundantly present intracellularly and is synthesized in the body from methionine and cysteine. However, in healthy animals it comes regularly from diets. In majority of animal's biological and physiological functions it several functions such as bile acid conjugation and prevention of cholestasis, heart functional effects, central nervous system neuromodulation, retinal development and function, endocrine/metabolic effects and antioxidant/anti-inflammatory properties (Lourenco and Camilo, 2002). This amino acid also protects endothelial layers and hence it is useful in maintaining the blood vessels intactness (Fennessy *et al.*, 2003). Alternatively, it serves as indirect agent in the maintainence of immune function and protects tissue or leukocytes from damage (Park *et al.*, 2001). Milk taurine has also been graded to be analgesic (Li *et al.*, 2005; Silva *et al.*, 1993).

Milk glutathione (GSH)

Glutathione is a triple peptide and it is a combination of sulphur amino acid cysteine, glycine and glutamic acid. It is profusely present in the cow's fresh milk and gets oxidized to form oxidized glutathione (GSSG). During oxidation, ROS gets removed and acts as antioxidant glutathione which regulates the production of insulin. ROS is known to inhibits the expression of pro insulin gene and therefore by inhibiting ROS it regulates glucose



balance. Glutathione, other than acting as growth factor, anti-apoptotic factor in leukocytes and cytokine secretion (Sprietsma *et al.*, 1999) and is also considered to be important in mitigating influenza through its central action on lungs. (Cai *et al.*, 2003).

A hypothesis that has been put forward during 1990's by RB Elliott and CNS McLachlan and collaborators that the protein from some cow's milk (not all the cows) raises risk for type I diabetes (DM-1) and coronary heart disease (HD), schizophrenia and autism as well. However, the protein that has been found so far in the cow's milk are A1 form of β -casein, and the most abundant protein in cow's milk are A1, A2 and β -casein.

Bovine milk and its products remained the main ingredient of human nutrition invariably. Consumption of milk and its products differs with different countries. For example, consumption of milk is considerably higher in Finland as compared to Japan and Korea (Saxelin *et al.*, 2003). Westernization has impacted the consumption during the last decades (Utviklingen, 2003). However, west relates this declining milk consumption due to the ill effects of milk and milk products. It was demonstrated that consumption of milk leads to heart diseases, weight gain and obesity (Insel *et al.*, 2004). The association between food and health was established in the year 2004 (Insel *et al.*, 2004) and many consumers in today's fora are highly aware of health-properties of food (Yusuf *et al.*, 2004).

Milk from cows are important food which gives us not only high quality proteins, but also carbohydrates and selected micronutrients. Almost all milk (95% around) is casein and whey proteins. Beta casein is abundantly present and considered as one of the excellent source of nutrition and amino acids. Mutations in the beta casein have given rise to 12 different genetic variants and out of these two A1 and A2 are the most common. Gastrointestinal proteolytic digestion of A1 variant of beta casein (raw/processed milk) leads to production of bioactive peptide, beta casomorphine 7 (BCM7) (Elliott *et al.*, 1999). BCM-7 may get absorb in the infants GIT as compared to adults. In A1 milk, BCM-7 level is 4 fold higher than in A2 milk. Moreover, the initial studies on indigenous cow (Zebu type), buffalo and exotic cows (taurine type) have shown that A1 allele is frequent in exotic cattle while Indian native dairy cow and buffalo have only A2 allele, (Mishra *et al.*, 2009) and hence considered as the source for safe milk.

Recently, a relationship between disease risk and consumption of either A1 or A2 milk. BCM7 causes human health hazards as it can potentially affect numerous opioid receptors in the nervous, endocrine and immune system. It is known to oxidize low dietary lipoproteins (LDL) and which forms arterial plaque. Few epidemiological studies demonstrated that consumption of beta-casein A1 milk associated as a risk factor for type 1 diabetes, coronary heart disease, arteriosclerosis and sudden infant death syndrome. At the same time other diseases such as autism, schizophrenia (Laugesen and Elliott, 2003; Tailford *et al.*, 2003) have also been shown to have associated with consumption of beta casein A1 milk. However, larger group researchers from west have shown reduction in autistic and schizophrenic symptoms with A1 milk intake (Cade *et al.*, 2000).

Consumption of milk with high levels of Beta casein A2 variant have been shown to witness lower incidences of cardiovascular disease and type-1 diabetes in a particular population.. It should be taken seriously and deeper research is needed to verify the range and nature of BCM7 interactions with the human gastrointestinal tract and whole organism.

Beta- casein polymorphism

There are 13 genetic variants of beta casein: A1, A2, A3, B, C, D, E, F, H1, H2, I, G. For the A4 allele, found in Korean native cattle, nucleotide substitution is not yet recognized. Various forms of beta β -casein in dairy cattle breeds are A1, A2, B, A3 and C (Farrell *et al.*, 2004) among these A1 and A2 forms are most common. In position 67 of the beta casein chain, proline in A2 is substituted by histidine in A1 (Groves 1969, Roginski, 2003).

Quantification methodology for assessment of beta-casomorphins

Milk and its products are matrices in which proteins, lactose, and lipids are intermingled and therefore interfere with separation and detection of target peptides producing erroneous quantifications. Reliable forms of methodologies are needed to be placed correctly to quantify them. Reversed-phase high performance liquid chromatography (RP-HPLC) are being used in separation of peptides and amino acids. For example, RPHPLC- UV

(Muehlenkamp and Warthesen, 1996) and ionexchange chromatography (Jarmolowska *et al.*, 1999) are being used for the separation and quantification of BCM7 present in cheese while HPLC-UV has been also used for determining BCM7 content in human milk (Jarmolowska *et al.*, 2007). Methods which uses UV- adsorption lacks sensitivity therefore requires quantification of even lower levels of BCM7 in dairy products. Quantification limit for BCM7 through RP-HPLC-UV is round about 2 mg/mL for cheese extract (Muehlenkamp and Warthesen, 1996). Despite these deficiencies, RPHPLC- UV is a well-established, relatively cheap, and has been regarded as user friendly analytical technique. Recently, HPLC along with mass spectrometry (MS) regarded as the best method for identification and quantification of peptides in the matrices of any dairy products. Various mass spectrometry techniques such as tandem mass spectrometry (MS/MS), quadrupole ion-trap mass spectrometry (QIT-MS), and time of flight mass spectrometry (TOF-MS) also helps in the identification of BCM's in the several dairy products (Table 1).

Table 1: Assessment methodologies of presence of beta-casomorphins in milk composed product and matrix

Sl.No.	Beta casomorphins	Composed product/matrix	Assessment methodology
1	BCM-3	Cheese	HPLC-UV
2	BCM-4	Cheese	HPLC-UV
3	BCM-5	Cheese	ELISA
4	BCM-6	Cheese	HPLC-UV
5	BCM-7	Cheese	ELISA
6	BCM-9	Yoghurt	LC-MS/MS
7	BCM-10	Cheese	HPLC TOF-MS, LC-MS/MS
8	BCM-11	Cheese	LC-MS/MS
9	BCM-11	Cheese and cow milk	HPLC-API-MS, Q-IT in full scan and MS
10	BCM-13	Yoghurt	Micro LC-TOF-MS

Tandem mass spectrometry allows accurate quantification of BCM5 and BCM7 at low levels (low mg/g) and hence considered very sensitive for investigation purposes. Similarly, QIT-MS has also been observed to quantify BCM5 and BCM7 in single reaction stroke. On the other hand, MALDI- TOF-MS preferably helps to search opioid-derived exogenous or endogenous peptides in

urines in cases of autism in human kids. HPLC, in addition also play important role in the search procedures when coupled with range of mass spectrometry technologies in quantifying BCMs in various dairy matrices even at lower levels (Cass *et al.*, 2008).

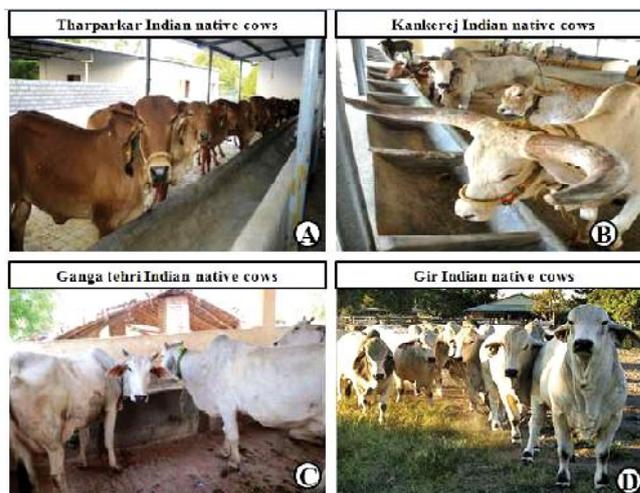


Fig. 2: Indian native breed originated from the different regions of the Indian peninsula

Diseases from the consumption of milk and milk protein

Hypothesis floated by RB Elliott and CNS McLachlan and collaborators during 1990s that a protein in the milk of some cows can cause type I diabetes (DM-I) and coronary heart disease (HD) (possibly also schizophrenia and autism). Such proteins have been designated as A1 form of β -casein. Some milk proteins have been identified as a source of active peptides-opioids (Brantl *et al.*, 1979; Chang *et al.*, 1985; Kostyra *et al.*, 2004). These opioids are chemically morphine type which could help in pain alleviation and controls the food intake. Such opioids binds to opioid μ -receptors in the central nervous system and the gastro-intestinal tract (Teschemacher, 2003). In their parent form such agents are inactive however, gets activated when digested in GIT or during the food processing etc. However, after the consumption of such dairy products when undergoes proteolysis in GIT also leads to the formation of various bioactive peptides (Kostyra *et al.*, 2002). Few studies have apparently demonstrated that A1 milk and its product posses risk for type 1 (insulin-dependent) diabetes mellitus (Elliott *et al.*,

1999) and ischaemic heart disease in humans (McLachlan, 2001). It has further been realised that that beta casein A1 yields the bioactive peptide beta casomorphine-7.

Elliott (1992) have stated that in type I DM the levels of cow milk antibodies raises and depending upon the data the group realizes that countries having higher consumption of milk has higher expressions of DM-1. Similarly, experiments on mice also illustrated that higher consumption of milk would lead to higher expression of diabetes in the population. Mice on diets having A1 -casein got diabetic but no diabetes occurred in the mice fed with A2 -casein. A bioactive seven-amino-acid peptide, -casomorphin-7 (BCM-7) can be released by digestion in the small intestine of A1 -casein with pepsin, leucine amino peptidase and elastase but the alternative proline at position 67 prevents a split at this site (Fig. 3).

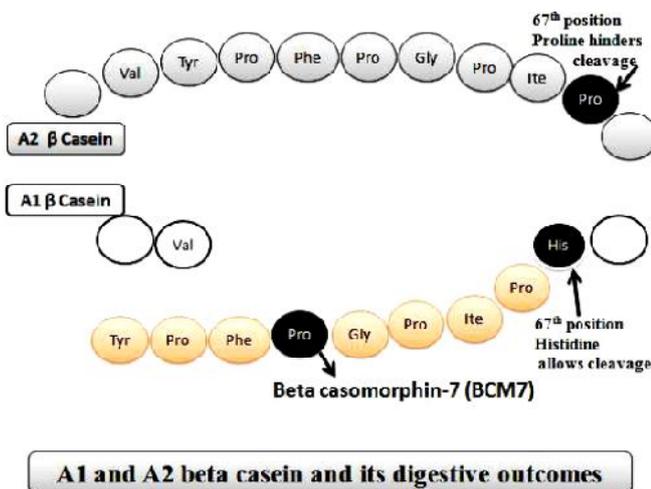


Fig. 3: Schematic representation of formation of beta casomorphine -7 with the breakdown of A1 milk chain of amino acid due to the presence of histidine at postion 67. However, amino acid chain remains intact in A2 milk amino acid chain due to the absence of histidine at position 67

BCM-7 has been shown and documented as an opioid and bio-modulatory product (Meisel, 2001). Synthetic BCM-7 can also inhibit responses of lymphocytes to stimulants *in vitro* (Elliott, 1992; Elliott *et al.*, 1997). BCM-7 is a bioactive (Shah, 2000) compound which considered as exomorphins (Meisel and FitzGerald, 2000) and have been shown to increase with more milk proteins in the diet. A1-derived BCM-7 has been shown to have a longer half-life (Panksepp *et al.*, 1984), reportedly detected in

urine (Cade *et al.*, 2000) of mice fed with A1 milk. This particular exomorphins have also been encoded in the human breast milk proteins but they are less active as compared to the bovine milk proteins (Herrera-Marschitz *et al.*, 1989). BCM-7 Peptides derived from caseins have also been shown to produce immunoreactivity (Svedberg *et al.*, 1985) and detected in human plasma (Chabance *et al.*, 1998). There are several reports that claims that the certain precursors of BCM-7 are also present in the plasma of newborn calves having consumed A1 milk from cows (Umbach *et al.*, 1985) but same thing did not happen in case in adults particularly dogs (Singh *et al.*, 1989). BCM-7 gets produced gets transmitted into circulation following digestion, notably in newborn or young mammals and easily detected the urine (Cade *et al.*, 2000) of patients with neurological conditions relying on casein free diet. Studies have also demonstration the transmission of BCM-7 through blood–brain barrier (Banks and Kastin, 1987; Sun *et al.*, 2003). Few studies have also shown traces of BCM-7 may cross the breast parenchyma–blood barrier into plasma and subsequently penetrate the blood–brain barrier to reach the central nervous system of the developing foetus (Nyberg *et al.*, 1989). It is alarming to note that it also causes apnea in infants due to the consumption of bovine milk of A1 origin which lead to sudden death in new borns (Sun *et al.*, 2003).

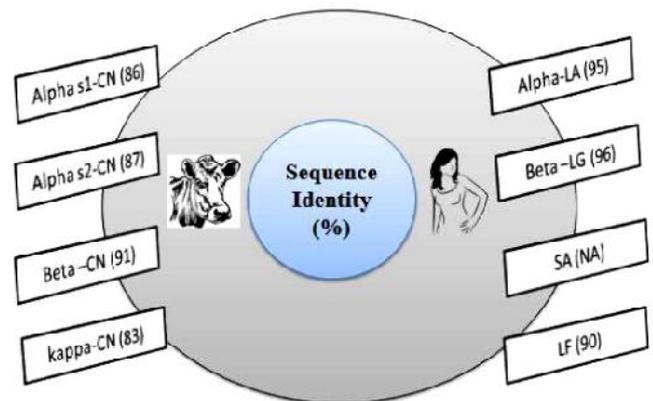


Fig. 4: Schematic presentation of comparison of sequences between cow and human milk peptides drawn from clustal W2 tool. Protein enteries were retrived from nrNCBI database

A1 milk has been found to be very common in dairy cows of north Europe such as Friesian, Ayrshire, British Shorthorn, and Holstein. On the other hand, A2 milk are

common in Island cows, Guernsey and Jersey, in Southern French breeds, Charolais and Limousin (Ng-Kwi-Hang and Grosclaude, 1992), and in the Zebu original cattle of Africa and Indian peninsula (Sahiwal, Tharparker, Ganga tehri).

Coronary heart disease arises with the consumption of A1 milk and thus it hints at the fact that there could be something present in such milk that raises or lowers the blood pressure or serum cholesterol. However, Truswell and his team have further declared that so far no plausible confirmation has been put forward that says that consumption of A1 milk raises the chances of CHD. Moreover, the BCM-7 has been linked already as an opiate receptor agonist (Meisel and FitzGerald, 2000) but also as a peptide with the ability to catalyse the oxidation of LDL in a non cation dependent fashion (Torreilles and Guerin, 1995). Oxidised LDL uptake by endothelium-bound macrophages leads to pathogenesis of atherosclerosis (Siow *et al.*, 1999). Thus it is found to be plausible biochemical mechanism relating to the relationship between A1 beta casein (not 'A1 milk') leading the production of (Tailford *et al.*, 2003; Steinerova *et al.*, 2004).

Moreover, the Truswell team had also meant earlier that milk protein devoid of A1 (A2-fed group) shows apparent attenuation of cholesterol-induced damage. Such results from Truswell team vouch for its ill-effects on the heart and its function (FitzGerald and Meisel, 2000; Scott, 1996). It was further noted that neurological problems gets potentiated due to the consumption of A1 milk which is being related directly to autistic spectral disorder (ASD) and schizophrenia (Knivsberg *et al.*, 2002).

CONCLUSION

Mutational procedures in bovine beta casein gene have given rise to 12 different genetic variants and among them A1 and A2 variants have been observed to be very common. The A1 and A2 variant differs at amino acid position 67 with histidine (CAT) in A1 and proline (CCT) in A2 (SNP difference). This single nucleotide polymorphism has caused a conformational change in the secondary structure of expressed β -casein protein. Intestinal degradation of A1 β -casein (raw/processed milk) produces bioactive peptides i.e. beta casomorphin 7 (BCM7) which gets digested soon in the infants and affects them more. Production of BCM-7 from A1 consumption is four fold higher in infants

compared to adults. A1 allelic frequency has been found to be more in exotic cows compared to the native dairy cows (Fig. 2) with hump. Moreover, it has been seen and observed that the population which consumed milk containing high levels of β -casein A2 variant have a lower incidence of cardiovascular disease and type-1 diabetes. However, the detailed investigation needs to be done in this direction to address the issue precisely.

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